Determination of Trace Element Stability in Sediments Using Redox Gel Probes: Probe Construction and Theoretical Performance

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A simple and inexpensive technique is described that can be used to assess the stability of redox-sensitive compounds in the sediments of wetlands and other shallow water environments. In this method, solid redox-sensitive compounds, such as manganese dioxide (MnO_2), are incorporated into agar gels held in rigid plastic holders. One surface of the gel remains exposed along the length of the resulting probe. The probes are pushed vertically into sediments and are left in situ for a period of time (days to weeks), after which they are visually inspected and chemically analyzed. The diffusion of nonreactive solutes (e.g., sulfate) in 2% (wt/vol) agar was unaffected by the presence of immobilized MnO_2 particles. The rate of dissolution of particulate MnO_2 in agar gels in the presence of an external diffusing reductant (L-ascorbic acid) could be quantified by digital analysis of pixel density on gel images. Redox gel probes incubated in the sediment of a wetland built to remove manganese from circumneutral pH coal mine drainage demonstrated different patterns of depth-dependent MnO_2 stability along a 15-m transect. MnO_2 gel probe results were consistent with data obtained using sediment cores and porewater diffusion samplers.

Keywords coal mine drainage, early diagenesis, manganese oxide, redox gel probes, sediments, wetlands

Introduction

The early diagenesis of redox-sensitive elements in freshwater and marine sediments results in their distribution with depth in a way that reflects the intensity of chemical and biological redox reactions, the diffusion of soluble chemical species, the rate of sediment accumulation,

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and other factors. A great deal of information can be discerned from sedimentary solid phase and porewater chemical profiles (Berner 1980), but the physical processing of sediment cores can be both time-consuming and expensive, especially when many cores must be taken to characterize a given area. In addition, the physical deformation of sediments during coring and sectioning operations often brings the relationship between cored material and the actual sedimentary environment into question (Morton and White 1997). Porewater samplers based on the diffusion of solutes across membranes into sampling chambers are tedious to prepare and sample, and often cannot provide the fine-scale vertical resolution necessary to detect transitions in the depth distribution of redox-sensitive elements (Hesslein 1976; Mayer 1976; Edenborn et al. 1986).

Alternative methods of studying diagenetic processes in sediments have been proposed to avoid some of the problems inherent in sediment and porewater sampling. One interesting approach is the use of relatively nondisruptive indicator sticks or probes to determine the location of zones of geochemical activity within sediments. Weidemann (1972) was one of the first to use this approach, and he used thin rods coated with red lead (Pb_3O_4) paint to detect and localize dissolved sulfide in wet and waterlogged soils. The rods were pushed vertically into the soils and left in place for 4 weeks, after which distinctive black zones of lead sulfide (PbS) formed on the rods where contact with free sulfides had occurred.

Reeburgh and Erickson (1982) also described a somewhat similar dipstick sampler for the examination of free sulfide profiles in estuarine sediments. Their samplers consisted of thin (ca. 2 mm) polyacrylamide gels containing lead acetate solution that were placed vertically in sediment and allowed to incubate for 15 min. The resulting distribution and intensity of gel color due to the formation of PbS precipitate correlated closely with the total sulfide concentrations measured chemically in the adjacent porewaters. This approach was somewhat analogous to the laboratory studies of Temple and Le Roux (1964), who demonstrated the precipitation of metal sulfides in agar gels separating sulfate-reducing bacteria and a source of diffusing heavy metal ions.

Aller and Rude (1988) also constructed prototype "authigenic mineral probes" to examine MnO_2 dissolution and transformation in sediments specifically. These probes were made by attaching MnO_2 particles to glass microscope slides with an epoxy glue. The slides were then inserted into marine sediments in the laboratory, and after several days of incubation, the transformation of MnO_2 to manganese-rich carbonates, such as kutnohorite ($Ca(Mn, Mg, Fe)(CO_3)_2$) and manganoan calcite, was detected by X-ray energy-dispersive microanalysis.

Recently, the research group of William Davison at Lancaster University (United Kingdom) has developed and extensively studied the performance characteristics of two different gel probes used to investigate trace metals and other components in sediment porewaters. The first of these methods, termed the "diffusive equilibration in thin films" (or DET) approach, relies on the diffusion of ions into a ca. 1-mm thick polyacrylamide gel held in a plastic probe and inserted into the sediment (Krom et al. 1994). After complete equilibration has taken place, the gels are recovered, sliced into thin sections, and analyzed. This method improved upon classic "peeper" dialysis methods by allowing a finer-scale resolution of porewater constituents, but porewater concentration profiles tended to "relax" due to the rapid lateral diffusion of constituents within the gels (Harper et al. 1997). This method has recently been redesigned to enclose gel segments within 200-um wide compartments in a 400-um thick ceramic sheet to eliminate this problem (Fones et al. 1998).

A second gel probe method designed and tested by the same group is termed the "diffusion gradients in thin films" (or DGT) approach. In this method (Davison and Zhang 1994), labile metal species in porewaters are immobilized in an exchange resin after diffusion across a polyacrylamide gel of known thickness (ca. 0.4 mm). The in situ flux of metals from

porewater to the resin can then be calculated based on the mass that accumulates per unit area during a known incubation period (Davison et al. 1994). This method establishes a defined diffusion gradient in the thin film under conditions of controlled mass transport, as opposed to the DET method, which requires that equilibrium with the porewater be established. Extremely fine-scale resolution of dissolved metals in sediments has been reported using this approach (Davison et al. 1997), and the technique has been applied to soils as well (Zhang et al. 1998).

In the present study, we developed a hybrid approach based on the various methods described here. In this method, redox-sensitive particulate compounds are immobilized in an agar gel matrix held in a grooved plastic rod, and these rods are inserted vertically into sediments. After incubation in situ, the rods are retrieved and the distribution of remaining particles is evaluated using either chemical, optical, or a combination of methods. This article describes the construction of these redox gel probes and the characteristics of their theoretical behavior and response under simulated and field conditions.

Materials and Methods

Redox Gel Probe Construction

Redox gel probes were prepared by mixing specific metal precipitates into a gelling solution, pouring the suspensions into prepared gel holders, and allowing the gel to solidify. This required the use of an appropriate gelling agent, the manufacture of appropriate gel holders, the synthesis of fresh metal precipitates, and the preparation of the final gel probes. In this description, the preparation of MnO_2-containing gel probes will be described, but other redox-sensitive particles can be substituted accordingly.

Assessment of suitable gelling agents for redox gel probes. Several materials were initially tested in wetland sediments for their utility as suitable gelling agents. These included 2% (wt/vol) carageenan (Sigma Chemical Co., St. Louis, MO, USA), 2% (wt/vol) gellan gum (Phytagel; Sigma Chemical Co.), 0.5% (wt/vol) low EEO electrophoresis-grade agarose (Fisher Scientific Co., Pittsburgh, PA, USA), three concentrations of purified agar (1, 1.5, and 2% (wt/vol), Fisher Scientific Co.), and silica gel. All gels except silica gel were prepared by heating in deionized water to boiling, dissolving the gelling agent, and followed by cooling to solidify the gel. Silica gel was prepared by mixing potassium silicate solution (10 g silica gel in 100 mL 7% [wt/vol] aqueous KOH, dissolved by heating) with a 20% (vol/vol) solution of *o*-phosphoric acid (85%, certified grade), as described for the preparation of bacteriological media (Krieg and Gerhardt 1994). Gel preparations were poured into gel holders (described later) and allowed to solidify. These gels were placed in wetland sediments and allowed to incubate in place for 15 days prior to further evaluation. Gels were evaluated based on their relative ease of preparation, handling characteristics, relative gel strength, resistance to abrasion during placement in sediments and subsequent survival in the field, and cost. Based on these criteria, 2% agar was chosen as the gel material used in all subsequent studies.

Gel holders. Gel holders (Figure 1) consisted of pointed Plexiglas rods (1 × 38 cm), each carefully milled with an engine lathe and ball-nose end mill to contain a single longitudinal groove approximately 34 cm long, 6 mm wide, and 3.5 mm deep, with a total volume of approximately 8 mL. Prototype versions of this gel holder were made out of disposable 10-mL polystyrene plastic serological pipettes (Kimble Science Products, 1 × 35 cm). One side of each pipette was ground down approximately one-third of the diameter of the pipette along most of its length, and the ends were plugged with silicone rubber cement. However,

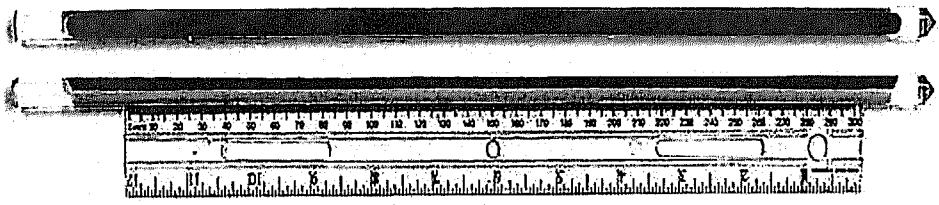

FIGURE 1 Top and side view of redox gel probes containing MnO_2 particles immobilized in a 2% (wt/vol) agar gel. Cross-sectional slices of gel are shown in Figure 4.

the final dimensions of these prototype holders were generally too irregular to provide gels of uniform shape and volume from holder to holder, and the brittle plastic with which they were made was more prone to breakage in the field.

Preparation of MnO_2 precipitate. Manganese dioxide precipitate was prepared by adding 500 mL of 20 mM $KMnO_4$ (adjusted to pH 12.4 with 1N NaOH) dropwise to a vigorously stirred solution of 1 L of 20 mM $MnSO_4 \cdot H_2O$. The resulting fine brown/black precipitate was mixed for an additional 15 min and allowed to settle. After 15 min, the supernatant was decanted, and the precipitate was washed with five 1.5-L volumes of deionized water in a similar fashion. Dissolved manganese concentrations in the final wash water were low (between 2 and 19 mg/L) and the final pH was between 6 and 7. The precipitate was stored in the final wash water prior to use. X-ray diffraction analysis (XRD) indicated that this precipitate preparation was initially amorphous, but became more crystalline within several days, giving variable but detectable XRD refraction patterns consistent with the manganese oxides akhtenskite (ε-MnO_2, hexagonal), ramsdellite (MnO_2, orthorhombic), hausmannite (Mn_3O_4, tetragonal), and birnessite ($Na_2Mn_{14}O_{27} \cdot 9H_2O$, monoclinic). Initial probe studies using crystalline chemical-grade pyrolusite as the particulate manganese oxide indicated that this compound was not readily reduced in situ, as also seen by Burdige et al. (1992), and the particles were too heavy to produce an appropriately homogeneous gel suspension during the agar-gelling step.

Redox gel probe assembly. A 2% (wt/vol) purified Bacto agar (Fisher Scientific Co.) solution was prepared in boiling deionized water and was allowed to cool to about 50°C in a heated water bath. MnO_2 precipitate slurries were then gravity filtered (Whatman #141 filter paper) until a wet paste was formed. The precipitate was weighed, added to the agar solution, and mixed with a magnetic stir bar to give a homogeneous 1% MnO_2 (wt/vol) suspension without introducing air bubbles. Eight mL of the suspension was then transferred by pipette to the longitudinal groove in the gel holders on a level surface and allowed to cool. The gel probes typically contained ca. 0.75 to 1.5 μmol total Mn cm^{-1} gel slice (ca. 65–130 μg particulate MnO_2), depending on the batch being prepared. After the agar had solidified (usually within 1 min), gel probes were used immediately in laboratory studies, or were stored under inert gas in plastic bags and were placed in the field within 4 h. On some occasions, gel probes were prepared in the field and were used immediately following their preparation. In specific experiments, control gels were removed from their holders immediately following gel solidification. These gels were sliced into 1-cm sections with a clean razor blade, digested in a 1:1 (vol/vol) mixture of concentrated HCl and HNO_3, brought to constant volume, and analyzed for total metals by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) on a Perkin Elmer Optima 3000 Radial-View spectrometer.

Laboratory Gel Studies

Experiments were carried out to examine the diffusional characteristics and chemical response of MnO_2 redox gel probes to ions in solution.

Sulfate diffusion in 2% agar gels with and without added MnO_2 particles. The rate of diffusion of a nonreactive ion in agar gels was examined. Plastic 5-cc syringes with the distal ends removed were filled with either 2% (wt/vol) agar or the same solution containing 1% (wt/vol) MnO_2 particles, as described previously. The exposed ends (ca. 113 mm^2) of three syringes of each gel type were placed into a stirred 1.4-L deoxygenated solution of 0.1 M potassium phosphate buffer (pH 6.8; KH_2PO_4/K_2HPO_4). Carrier-free ^{35}S sulfate (ICN Pharmaceuticals, Irvine, CA, USA) was added to the solution to a final activity of 2.5×10^5 dpm/L, and the experiment continued for 21 h at room temperature (20°C). After that time, the gel cylinders were extruded, sliced into layers ca. 1.35 mm thick (1.53 cm^3), and placed into scintillation vials containing 10 mL of liquid scintillation cocktail (EcoLume, Fisher Scientific Co.). After an equilibration time of 24 h, these were assayed for radioactivity in a Pharmacia 1214 RackBeta liquid scintillation counter. Data were analyzed mathematically as described in the Results section.

Effect of L-ascorbic acid on MnO_2 stability in gel probes. The effect of various concentrations of ascorbic acid on the dissolution of MnO_2 particles immobilized in gel probes was investigated by preparing standard 1% (wt/vol) MnO_2 gel probes in holders as described previously. These were placed in aqueous solutions containing 0, 1×10^{-3}, 2.5×10^{-3}, 5×10^{-3}, 7.5×10^{-3}, and 1×10^{-2} M L-ascorbic acid (tissue culture grade; Fisher Scientific Co.) buffered to a pH of 7.0 ± 0.1 with 10 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Sigma Chemical Co.). Gels were placed in the various solutions for 1 h, after which they were removed, photographed as described later, and sliced into 2-cm sections. Replicate gel slices were extracted for 3 h with oxygen-free 10 mM $CuSO_4$ solution (pH 4.5), digested in a 1:1 (vol/vol) mixture of concentrated HCl and concentrated HNO_3, brought to constant volume, and analyzed for total manganese by ICP-OES. A similar experiment designed to examine pixel density in reacted gels was carried out under identical conditions, but using concentrations of 0, 10^{-3} M, 10^{-2} M, and 10^{-1} M L-ascorbic acid in 10 mM Hepes buffer.

MnO_2 particle density distribution in various gels was determined by photographing them under conditions of identical transmitted light and magnification settings with a Leica Wild M3Z microscope equipped with a Spot digital CCD camera (model 1.3.0; Diagnostic Instruments, Sterling Heights, MI, USA). The images were captured with PAX-it! software (MIS, Inc., Franklin Park, IL, USA). The gel images were subsequently digitized and analyzed using the Un-Scan-It gel for Windows program (version 5.1; Silk Scientific, Orem, UT, USA). Depending on the gels analyzed, the lane analysis mode in this software package was used to scan for changes in pixel density with distance on gel images, or the segment analysis mode was used to add all pixels within an identical area on different gel images for comparative purposes. MnO_2 calibration curves for pixel density standardization were prepared by serially diluting a 2% (wt/vol) molten agar solution containing 1% MnO_2 (wt/vol) in additional 2% agar prior to preparing gel probes as described previously.

Field Experiments

A few preliminary experiments were conducted in a wetland located in northwestern Pennsylvania (41°08′29″N, 79°29′21″W). The wetland system was built by a mining company to remove manganese from a complex mixture of alkaline drainage from an underground coal mine and acidic water draining from spoil piles. For the purposes of this initial

research, the wetland site was used to see what kinds of reactions were observed in MnO_2 gel probes left in wetland sediment, and to determine how these reactions compared with traditional methods of sediment and porewater analysis.

Field deployment of redox gel probes. MnO_2 gel probes were placed in the sediment at 1-m intervals along a surveyed 15-m north-south transect within the wetland. Surface water depth and sediment/substrate thickness at each sample point were both measured. Substrate thickness was defined as the depth below the sediment/water interface that could be manually penetrated using a calibrated 2.5-cm diameter PVC pipe (Stark and Williams 1995). Each probe was placed vertically into the sediment so that approximately 4 cm of the agar gel (of a total 34 cm) remained above the sediment-water interface, facing perpendicular to the flow of water, where discernible (Figure 2). The height of the gel probe initially extending above the sediment surface was noted as a check on any further sediment deposition during the incubation period. Care was taken to avoid disturbance of the sediment near the probe insertion locations. Length of incubation time required in a given environment is dependent on the sedimentary characteristics at a given site. Preliminary tests at the study wetland

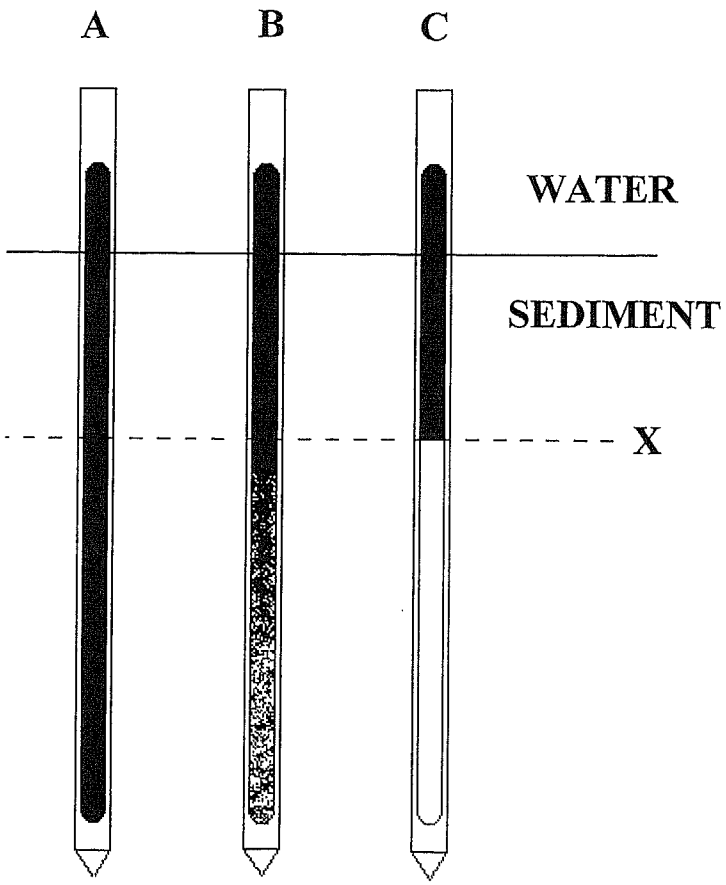

FIGURE 2 View of MnO_2-containing gel probes in place in sediment. Gels are placed vertically in sediment with some gel extending above the sediment-water interface. Dashed line represents the hypothetical sediment depth below which the immobilized solid compound is unstable. The gels shown represent their appearance after (A) initial placement, (B) intermediate incubation time, and (C) after maximum incubation time in situ.

showed that 7 days was sufficient to produce visually detectable changes in gel precipitate characteristics (data not shown). In this study, the gels were removed after 21 days of incubation, and the visual changes in the appearance of the incorporated MnO_2 particles were observed and recorded. Four basic reactions based on particle density or color change were observed and are described further later. The depth intervals over which these reactions occurred were measured relative to their distance from the sediment-water interface at the time each gel probe was removed from the sediment.

At a site adjacent to the transect described previously, interstitial porewater samples were collected using an equilibrator/peeper similar to those described by Hesslein (1976). The equilibrators are 64-cm-long Plexiglas stakes (6.5 × 6.5 cm) containing 24 wells (1.4 × 4.7 × 5 cm) of ca. 33 mL volume spaced evenly along the length of the stake. The wells were filled with deionized deoxygenated water and covered with a dialysis membrane (Spectra/Por 1; 6,000–8,000 Da molecular weight cutoff; Spectrum Laboratories, Rancho Dominguez, CA, USA). The stakes were pushed down into the sediment of the wetland and allowed to equilibrate with the interstitial water for 21 days. Immediately upon retrieval, replicate 10-mL samples were taken from each well, passed through 0.45-μm pore size syringe filters (SFCA membrane; Corning, Inc., Corning, NY, USA), and acidified with concentrated HCl prior to chemical analysis.

Sediment cores were collected by manually inserting 5.5-cm-diameter Plexiglas core liner tubes into the sediment and sealing the bottoms with rubber stoppers by hand prior to removal. A simple piston was used to extrude the sediment core upward after surface water had been siphoned off, and 1-cm core sections were collected as the sediment was exposed. The outside layer of each section was removed to eliminate contamination due to core smearing. Sediment sections were then placed in clean water sample bottles and frozen on dry ice prior to further analysis. Sediment samples for particle size determination and total metals analysis were oven-dried at 105°C to a constant weight. Samples for metals analysis were digested in a 1:1 (vol/vol) mixture of concentrated HCl and concentrated HNO_3 prior to analysis by ICP-OES. Data were not corrected for the possible influence of core shortening (Morton and White 1997).

In one experiment, the wetland sediment was sampled by coring, and the surficial 0- to 1-cm-depth interval was collected. This material was immediately added to a molten 2% (wt/vol) agar solution prepared in the field. Gel probes were prepared from this mixture and were placed in the sediment adjacent to MnO_2 probes (2.5 cm) prepared as described previously. After 21 days, the probes were removed from the sediment and cut into 1-cm sections with a clean razor blade. Gel slices were digested in a 1:1 (vol/vol) mixture of concentrated HCl and HNO_3, brought to constant volume, and analyzed for total manganese by ICP-OES.

Analytical Methods

Measurements of pH were made using an Orion SA270 portable field meter and a combination pH electrode using a two-point calibration (Thermo Orion, Beverly, MA, USA). Redox potential was determined with an epoxy-body redox platinum combination electrode (Thermo Orion) standardized with solutions containing potassium ferrocyanide and potassium ferricyanide. Alkalinity (as $CaCO_3$ equivalents) and acidity were determined titrimetrically using U.S. Environmental Protection Agency (EPA) methods 310.1 and 305.1, respectively (U.S. EPA 1983). Sulfate concentrations were analyzed by barium chloride titration, using thorin as the endpoint indicator, following passage of the raw sample through a cation exchange column. All other ions were determined by ICP-OES using U.S. EPA method 200.7. The percent recovery of calibration check standards typically had a standard

deviation <2% or less per element. The U.S. EPA quality control guideline of one duplicate, one standard recovery, and one spike recovery for every 10 analyses was employed (U.S. EPA 1983). X-ray diffraction analyses were done using a Rigaku Geigerflex powder diffractometer (Rigaku/MSC, The Woodlands, TX, USA) and Jade 3.1 software (Materials Data, Livermore, CA, USA).

Results

Diffusion Characteristics of Agar Gel Probes

The mathematical model for the current gel probe data is based on one-dimensional Fickian diffusion of nonreacting sulfate ion through a semifinite gel, which is initially at zero solute concentration and whose surface is maintained at a constant solute concentration, i.e.

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2}, \quad x > 0$$
$$C(x, 0) = 0; \quad C(0, t) = C_0 \tag{1}$$

$C(x, t)$ is the time-dependent concentration of sulfate at point x in the gel; and D is the diffusion coefficient, taken as a constant. The solution to equation 1 is (Carslaw and Jaeger 1959):

$$\frac{C}{C_0} = 1 - erf\left[\frac{x}{2\sqrt{Dt}}\right], \tag{2}$$

where *erf* is the well-known error function, whose tabulated values can be found in most books of mathematical tables. This is the same expression used by Davison et al. (1994) to describe the diffusion of metal ions in DET polyacrylamide gel probes. Curve-fitted experimental $C(x)$ versus x data at a given time can yield a value for the diffusion coefficient.

For experiments involving the diffusion of radiolabeled sulfate into 2% agar gels, a plot of C/C_0 (*exptl.*) versus C/C_0 (*theor.*), calculated for various values of D, was used to evaluate the best fit (Figure 3). In this case, $D = 1.1 \times 10^{-5}$ cm^2 sec^{-1} was found to result in a good representation of the data over the range of the experimental values. Experiments done under identical conditions with gels containing 1% (wt/vol) MnO_2 particles yielded approximately the same diffusion rate for radiolabeled sulfate (data not shown).

Effect of Ascorbic Acid on MnO_2 in Gel Probes

Figure 4 shows the effect of different concentrations of L-ascorbic acid on MnO_2 particles immobilized in 2% agar gel probes after 1 h of incubation. The progressively increased solubilization of the MnO_2 particles and more advanced reaction front with increasing reductant concentration is evident in these gel cross-sections. Analysis of total manganese remaining in gel sections following this reaction, after leaching with 10 mM $CuSO_4$ to remove soluble manganese, revealed a linear relationship between the molar concentration of reductant and the total manganese remaining (Figure 5). The initial rapid drop in total manganese concentration noted between 0 and 1 mM L-ascorbic acid addition is likely due to initial reaction with MnO_2 at the gel surface and immediately subsurface, after which reductant transport was diffusion limited. In addition, a linear and positive correlation was

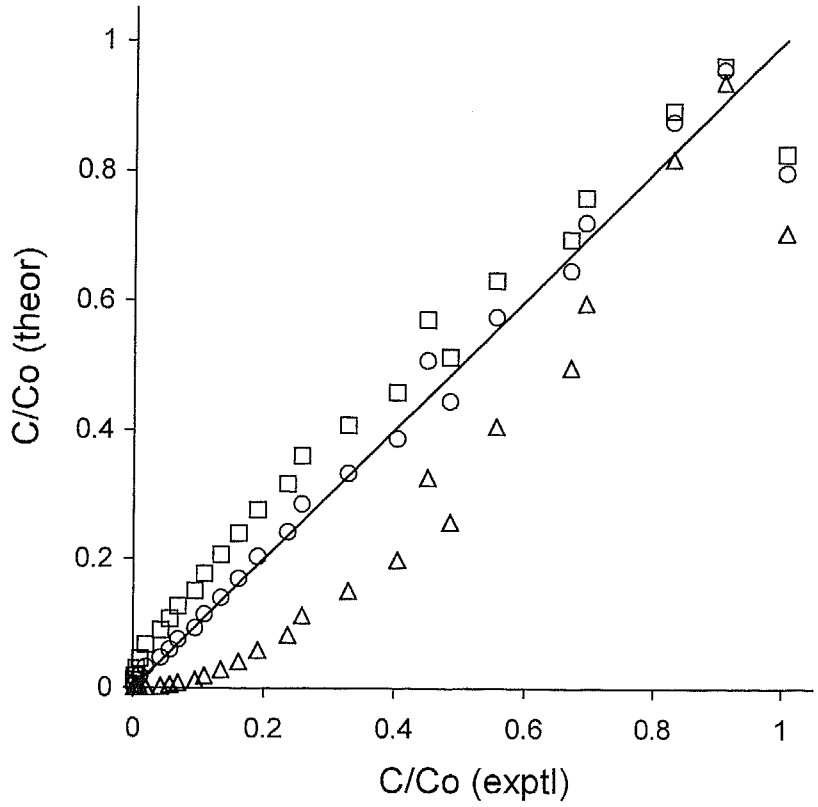

FIGURE 3 Evaluation of various diffusion coefficients for radiolabeled sulfate in a 2% agar gel, curve-fit to the experimental versus calculated data. Plots are shown for $D = 1.1 \times 10^{-5}$ cm^2 sec^{-1} (open circles), $D = 1.5 \times 10^{-5}$ cm^2 sec^{-1} (open squares), and $D = 0.5 \times 10^{-5}$ cm^2 sec^{-1} (open triangles), evaluated at $t = 80{,}000$ sec, with an effective $C_0 = 250{,}000$ dpm/ml.

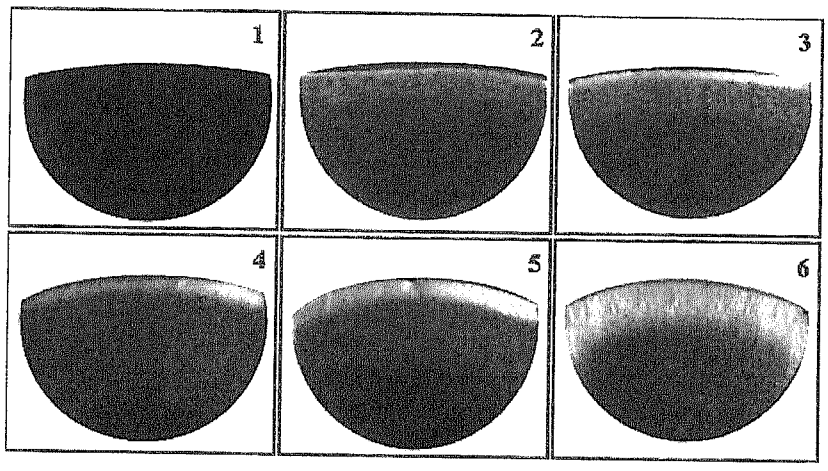

FIGURE 4 Photographs of cross-sectional slices of MnO_2 gel probes after incubation in various concentrations of L-ascorbic acid in 10 mM Hepes buffer (pH 7.0) for 1 h at room temperature. Gels were incubated in buffer only (1), and the following concentrations of L-ascorbic acid solution: 1×10^{-3} M (2), 2.5×10^{-3} M (3), 5×10^{-3} M, (4) 7.5×10^{-3} mM (5), and 1×10^{-2} mM (6).

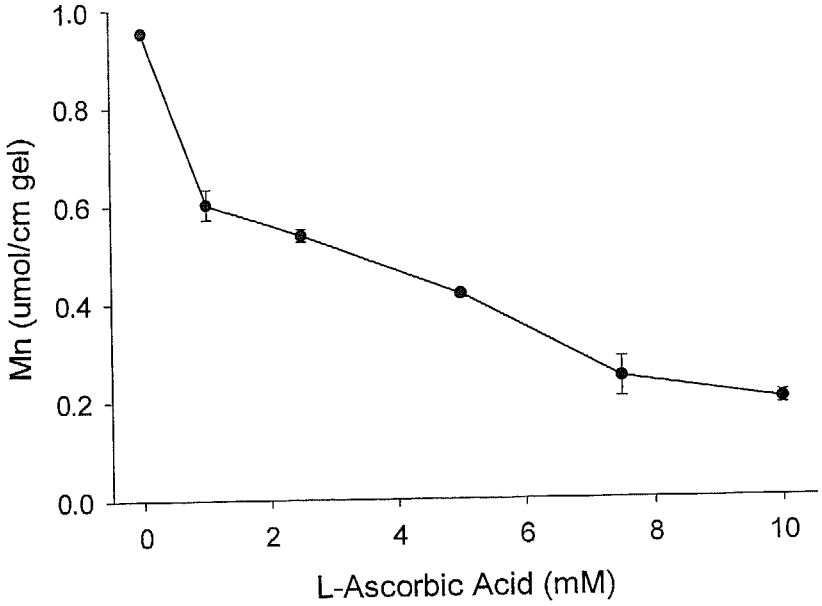

FIGURE 5 Total manganese remaining in MnO_2-containing gel probes after incubation with different concentrations of L-ascorbic acid at room temperature in 10 mM Hepes buffer (pH 7.0) for 1 h, and following extraction of soluble manganese with a 10 mM $CuSO_4$ solution. The results are the means of triplicate measurements. Error bars designate one standard deviation.

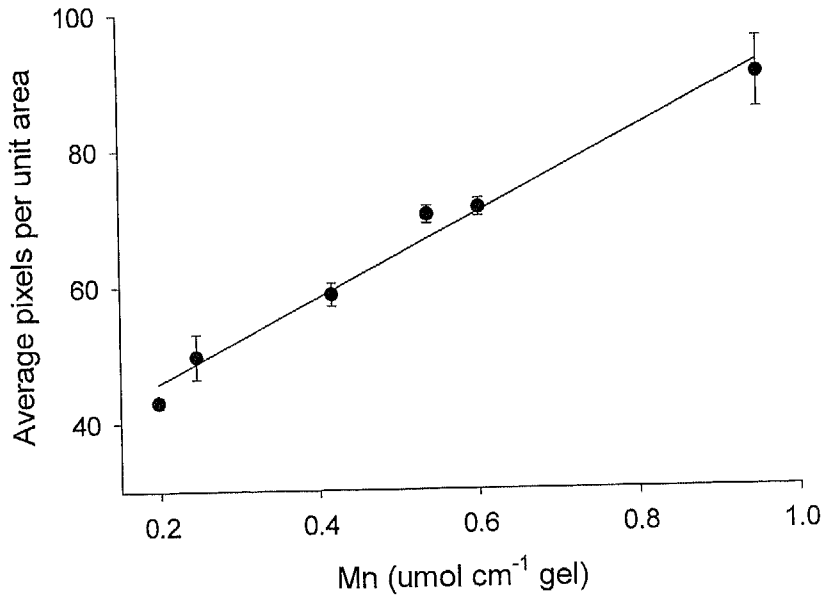

FIGURE 6 Gel image pixel density (minus background pixel density) of MnO_2-containing gel probes compared to total manganese content. The results are the means of triplicate measurements. Error bars designate one standard deviation.

observed for total manganese (as MnO_2) and the pixel density of digitally photographed images of the gels (Figure 6). MnO_2 gel probes placed in solutions of increasing L-ascorbic acid concentration were incubated for 1 h and then photographed at the air/solution (meniscus) boundary. As seen in Figure 7, the pixel density of the gels in solution was inversely related to the L-ascorbic acid concentration in solution. Dissolution of the MnO_2 particles was observed to occur above the meniscus boundary as well, due to upward diffusion of the reductant. This is relevant to the ability of the probes to resolve a true concentration gradient external to the gel. The impact of this upward diffusion resulted in a vertical resolution error of ca. 2 mm in distance in this case.

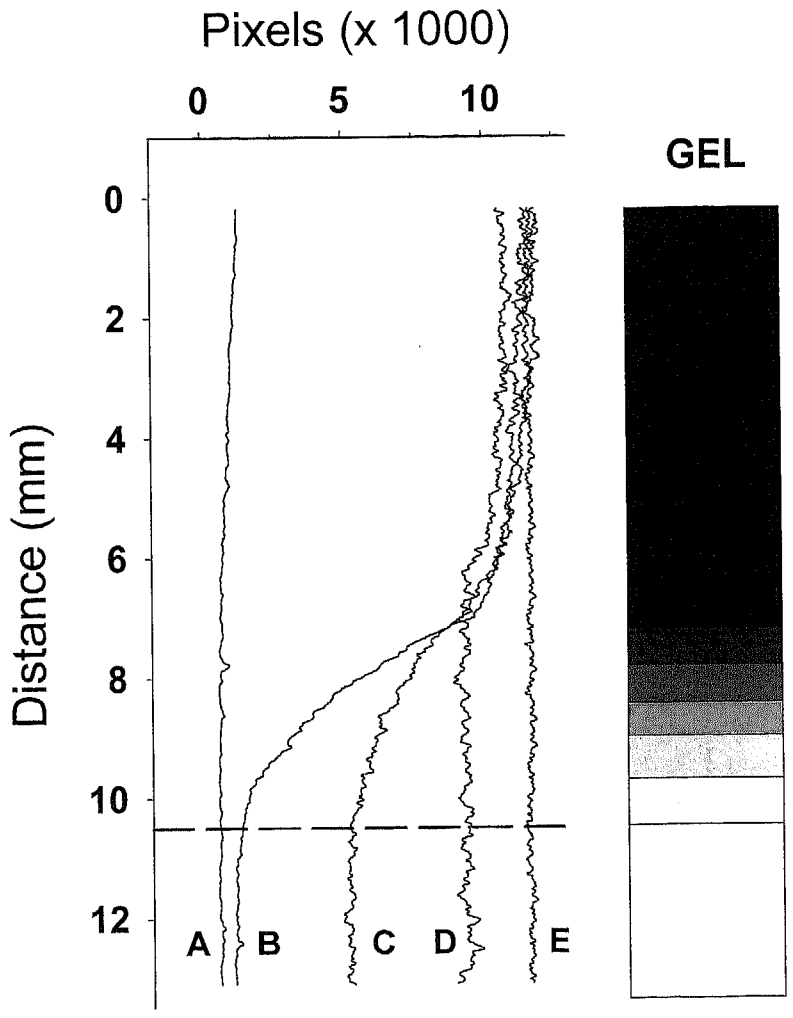

FIGURE 7 Pixel density scans of MnO_2 gels incubated for 1 h at room temperature in different concentrations of L-ascorbic acid in 10 mM Hepes buffer (pH 7.0). Gels were photographed in the ca. 1 cm long region encompassing the solution meniscus (dashed line). A generalized diagram of the observed transition from unreacted to reacted gel in this region for the more reducing solutions is shown at right. Scans include gel probe sections with (A) no MnO_2 added, as well as those incubated in (B) 1×10^{-1} M L-ascorbic acid, (C) 1×10^{-2} M L-ascorbic acid, (D) 1×10^{-3} M L-ascorbic acid, and (E) buffer only.

Field Studies

General Characteristics of the Wetland Water and Sediments

Briefly, the wetland effluent had a pH of between 6 and 7 and typically contained high concentrations of sulfate (ca. 1,800 mg/L), calcium (ca. 380 mg/L), magnesium (ca. 250 mg/L), manganese (15 mg/L), and very little dissolved iron (<1 mg/L). The wetland sediments consisted of the decomposing mulched hay and spent mushroom compost used in the initial construction of the wetland treatment system, as well as accumulated sand, silt, and vegetative debris. Typical particle size distribution for this sediment was 8 to 11% coarse sand, 21 to 28% medium sand, 50 to 59% fine sand, and 7 to 12% silt or clay (S. L. Edenborn, unpublished data). Sediment porewaters typically showed increasing alkalinity and pH over 0 to 30 cm depth, with decreasing redox potential and sulfate, calcium, and magnesium concentrations. Detailed data on the chemical characteristics of the surface and sediment porewater of this wetland are given in the accompanying article in this issue (Edenborn and Brickett 2002).

Response of MnO_2 Gels Incubated in Wetland Sediments

Three different transformations were visible in MnO_2 gel probes left for 21 days in the wetland sediment (Figure 8). The original brown/black MnO_2 precipitate in the gel was 1) reduced and solubilized incompletely, leaving some particulate MnO_2 remaining in the

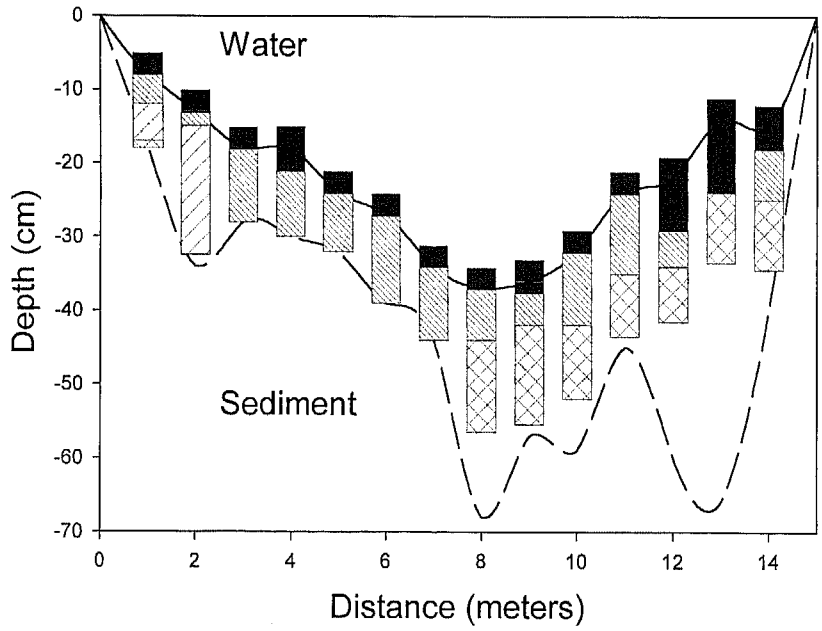

FIGURE 8 Response of MnO_2 redox gel probes incubated for 21 days along a 15 m (S to N) transect in the study wetland. The stacked bars indicate the reactions observed in the redox gel probes: black equals unreacted MnO_2; downward right diagonal equals incomplete dissolution of MnO_2; downward left diagonal equals complete dissolution of MnO_2; cross-hatching equals white precipitate. The lower dashed line represents the approximate lower limit of the organic substrate thickness. Note exaggerated vertical scale relative to the horizontal distance.

gel; 2) reduced and apparently solubilized completely, leaving a clear gel; or 3) reduced completely, with some or all of the original MnO_2 being replaced by a white precipitate. Other gels or gel sections often appeared visually identical to the original gels before their placement in the sediment. Wet chemical analyses showed that the white precipitate that formed in some gels was both warm acid- and carbon disulfide-insoluble, and thus unlikely to be either $MnCO_3$ or elemental S, respectively (Dana and Ford 1966). XRD analyses of this material indicated that it was gypsum ($CaSO_4 \cdot 2H_2O$). A fourth type of reaction was also observed where residual MnO_2 particles within the incompletely solubilized regions of the gels turned orange. Total metals analysis of acid-digested gel sections revealed that the orange particles in the gel contained predominantly iron, with only residual amounts of manganese.

Comparison of Reactivity of Model MnO_2 Particles and Manganese in Surface Sediments

The vertical profiles of total manganese in redox gel probes constructed with laboratory-synthesized MnO_2 and surface sediments from the study wetland after incubation in situ for 21 days are shown in Figure 9. The two profiles are similar in shape, despite the five-fold difference in total manganese concentrations in the two types of gel probes. This is somewhat surprising, in that labile carbon might be expected to have been incorporated within the gel as well, and possibly lead to more reduced conditions within the sediment probe. However, the similar profiles do suggest that synthesized MnO_2 serves as a useful surrogate in describing the geochemical behavior of sedimentary manganese oxides in this environment.

Comparison of Gel, Equilibrator, and Core Data

At one site in the study wetland, the stability of MnO_2 in a redox gel probe was compared and contrasted with the information that could be determined from equilibrator porewater and sediment core data (Figure 10). Although the decreasing porewater concentrations with depth in the most surficial sediments imply a downward diffusion of manganese into sediments from the surface water, the poorer depth resolution of the equilibrator data makes their relationship to the solid phase profiles difficult to interpret. Nevertheless, increasing

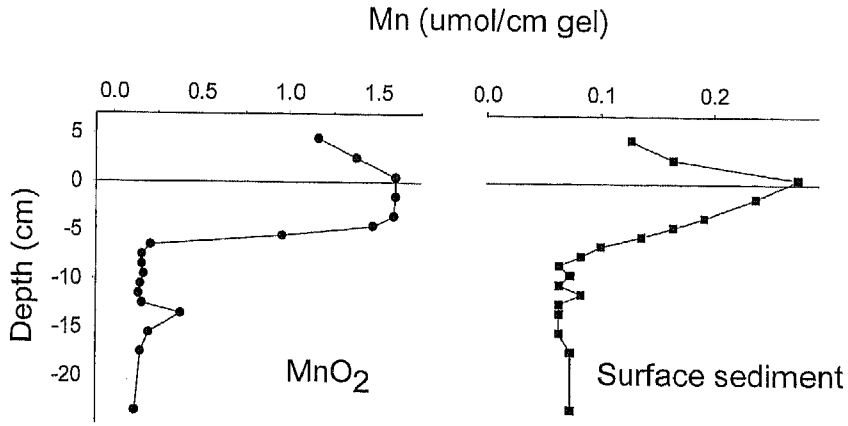

FIGURE 9 Vertical profiles of total manganese in gel probes containing MnO_2 (circle) or surficial wetland sediment (square) after incubation for 21 days. Gel probes were placed 3 cm from each other, with the exposed gels facing in opposite directions.

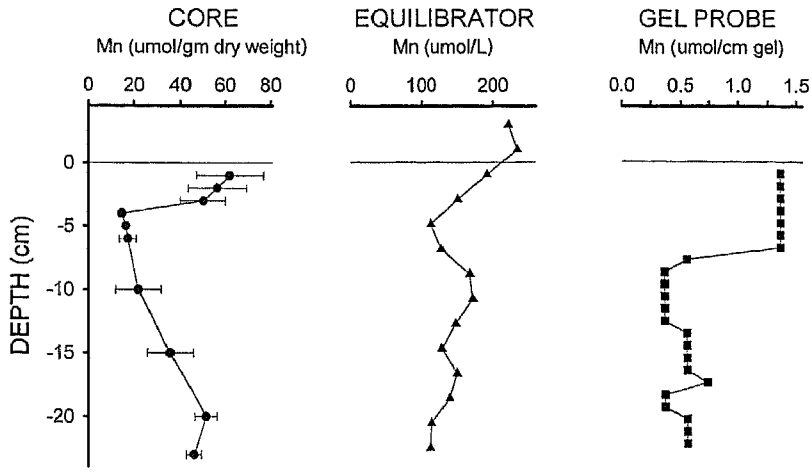

FIGURE 10 Comparison of total manganese in a sediment core, porewater manganese, and residual MnO_2 in a redox gel probe sampled within a 10-cm^2 area of the study wetland. The equilibrator and gel probe were left in place for 21 days, at which time the sediment core was taken.

porewater manganese concentrations upward and downward from ca. 5 cm depth are consistent with the apparent "stability interfaces" indicated by both the sediment core and probe data. Redox gel probe data indicated that newly introduced MnO_2 remained stable down to approximately 7 cm depth below the sediment-water interface after 21 days of incubation, whereas the total manganese concentrations in the sediment core decreased rapidly below ca. 3 cm. The similar dissolution profiles for sedimentary and synthetic manganese incubated concurrently in situ (Figure 9) suggest that MnO_2 stability in redox gel probes may be more reflective of current oxidizing conditions deeper in the surface sediment. In contrast, solid Mn concentrations in sediment cores are more likely to provide a record of accumulation and dissolution occurring over a longer and indeterminate period of time.

Discussion

The results of this study suggest that redox gel probes can be used as sensitive indicators of the environmental conditions affecting MnO_2 stability in a wetland sediment. Technical concerns exist regarding how precisely and accurately gels reflect the environmental conditions in adjacent porewaters (Harper et al. 1997). However, field data obtained regarding MnO_2 stability using redox gel probes in this study were not contradicted by information obtained using the more labor-intensive sampling procedures of equilibrator porewater sampling and sediment coring. Each of these latter techniques provides unique geochemical information that may be required as well at times (e.g., porewater profiles of multiple dissolved ions, evidence for metal retention in sediments, etc.). However, the redox gel probe technique provides a simple method that can be easily manipulated to provide information regarding the stability of a specific solid compound. The technique also potentially allows the field assessment of a given area using an almost unlimited number of sampling points, avoiding the sediment disturbance and lab time required to prepare and process samples using the other two methods. This approach can be especially valuable in the identification of environmental "hot spots" prior to more extensive sampling using conventional approaches. The preliminary in situ gel probe responses suggested that strong spatial variations exist in the thickness of zones of MnO_2 stability and dissolution in the sediment over a relatively short

distance (Hunt et al. 1997). This is in agreement with the recent results of Shuttleworth et al. (1999), who observed large differences in the porewater profiles of dissolved manganese and iron just 3 mm apart in seasonally anoxic lake sediments.

The particulate MnO_2 incorporated in the redox gel probes was intended to closely mimic the behavior of manganese oxides found in the sediment. However, these compounds may include a wide range of oxides and hydroxides of variable crystal structure and chemical composition (Murray et al. 1984; Burdige et al. 1992). Hem and Lind (1983) found that manganese oxides formed in aerated solution were metastable and altered by irreversible processes to more highly oxidized species during aging. The MnO_2 used in our studies was found to consist principally of akhtenskite, a natural analog of ϵ-MnO_2, that is believed to be formed biogenically (Chukhrov et al. 1989). Studies on the speciation of manganese in sediments of a similar natural wetland receiving mine drainage in the same area of Pennsylvania found that most of the manganese (~70%) was retained in the oxic surface sediments as potentially reducible, oxide-bound precipitates (Tarutis et al. 1992). The similar stability profiles with depth for surface sediment manganese and freshly precipitated MnO_2 (Figure 9) suggest that the synthesized material was a satisfactory surrogate for natural manganese oxide minerals formed in this particular environment, despite the expectation that the latter material would tend to become more crystalline with time.

One unusual situation created by the use of a gel to immobilize initially solid indicator compounds is that products of the reaction between these and soluble diffusing chemical species accumulate temporarily in the pore space of the gel. This may result in conditions where chemical concentrations become saturated or supersaturated with respect to a precipitate not even found in the environment adjacent to the gel. For example, we suspected that the acid-insoluble white precipitate forming within some MnO_2 gels at depth in the sediment was elemental sulfur, formed by the oxidation of sulfide (Burdige and Nealson 1986):

$$MnO_2 + HS^- + 3H^+ \rightarrow Mn^{2+} + H_2O + S^o$$

However, subsequent XRD identification of the white precipitate as gypsum suggests the potential oxidation of sulfide to sulfate (Aller and Rude 1988) by the incorporated MnO_2 particles,

$$4MnO_2 + HS^- + 7H^+ \rightarrow 4Mn^{2+} + SO_4^{2-} + 4H_2O,$$

followed by the supersaturation of gypsum due to the increased interstitial gel concentration of sulfate:

$$Ca^{2+} + SO_4^{2-} + 2H_2O \rightarrow CaSO_4 \cdot 2H_2O$$

This hypothesis was tested using the geochemical modeling program MINTEQA2/PRODEFA2 (Allison et al. 1991) by incrementally increasing the concentration of dissolved sulfate in anoxic wetland porewater with the following representative characteristics: pH (7.3), Ca^{2+} (300 mg/L), Mg^{2+}(65 mg/L), Mn^{2+}(6 mg/L), K^+ (11 mg/L), and Na^+ (15 mg/L). Calculations showed that the porewater became supersaturated with respect to gypsum when sulfate concentrations rose above approximately 1,600 mg/L. Adding alkalinity to this model porewater resulted in the precipitation of rhodochrosite, calcite, and dolomite. Formation of the latter two minerals lowered the concentration of porewater calcium and increased the amount of sulfate needed to precipitate gypsum (e.g., 4,000 mg/L sulfate in the presence of 210 mg/L Ca^{2+}). However, this is consistent with the hypothesis

that gypsum formation within the gel matrix probably occurred at depths where free sulfide was available for reaction with MnO_2, sufficient Ca^{2+} was present, but the porewater sulfate concentration was below that required for gypsum precipitation in porewater. The oxidation of dissolved sulfide by MnO_2 could then effectively raise the sulfate concentration in the interstitial space of the gel above that required for gypsum precipitation. Similar mechanisms have been proposed for the periodic precipitation of inorganic salts ("Liesegang rings") formed by one or more reactive solutes diffusing through gel media (Stern 1954; Wimpenny 1982). Reactive solutes also tend to be depleted in concentration in the zones adjacent to the bands of precipitation. An additional source of calcium for the formation of gypsum may be provided by Ca^{2+}adsorbed by the MnO_2 particles (Eley and Nicholson 1993), a process that may also account for the apparent surficial coating or replacement of the MnO_2 particles by gypsum. However, the particular juxtaposition of oxidized (MnO_2) and reduced inorganic species (S^{2-}) is unlikely to commonly occur in nature in locations other than specific environmental redox interfaces, and we likewise know of no previously reported pseudomorphs of gypsum after MnO_2.

A second reaction that occurred within some gel sections at depth in the sediment was the apparent partial coating or replacement of MnO_2 particles by iron oxide minerals. DeVitre et al. (1988) showed that MnO_2 precipitated from lake water was readily reduced in the presence of excess Fe(II), probably via the reaction:

$$2Fe^{2+} + MnO_2 + H_2O \rightarrow Fe_2O_3 + Mn^{2+} + 2H^+$$

In similar laboratory experiments (Golden et al. 1988), the synthetic manganese oxide minerals todorokite ((Mn, Ca, Mg) $Mn_3O_7 \cdot H_2O$) and birnessite ($Na_4Mn_{14}O_{27} \cdot 9H_2O$) acted as oxidizing agents toward Fe(II) in solution, precipitating Fe(III) as iron oxides that were pseudomorphic after the original manganese minerals. Complex intermediate solid phases, such as ferrihydrite ($Fe(OH)_3$) and jacobsite ($MnFe_2O_4$), can form on MnO_2 mineral surfaces undergoing reductive dissolution under simulated condition of acid mine drainage, effectively decreasing the rate of MnO_2 dissolution (Villinski et al. 2001). Sections of MnO_2 redox gel probes that turned orange during incubation in the wetland sediment contained an approximate 5:1 molar ratio of Fe/Mn (data not shown), implying that most of the initial MnO_2 was ultimately replaced by iron. The immobilization of MnO_2 particles in agar gels may also contribute to the formation of the apparent complete replacement of the particles by iron oxides. Good pseudomorphism of iron after MnO_2 typically requires that Fe(III) hydrolysis be rapid and no migration of Fe(III) from the site of the reaction occur (Golden et al. 1988). The visual orange discoloration of the MnO_2 particles could potentially serve as a useful indicator of ferrous iron distribution in sediment porewaters as well.

In practice, redox gel probes allow the titration of a common amount of the incorporated solid redox sensitive compound by the sum of all potential oxidizing or reducing compounds at each depth interval over the length of the probe. As such, they are highly *nonspecific* in their determination of agents active in the dissolution of the model compound. A wide variety of compounds is known to be capable of reducing manganese oxides, including humic type organics, Fe^{2+}, HS^-, phenols, As^{3+}, oxalate, pyruvate, succinate, biotic enzymes, and others (Stone 1987; Hamilton-Taylor and Davison 1994). Stone and Morgan (1984) examined the effects of 27 aromatic and nonaromatic compounds on the reduction of manganese oxide suspensions, and found that ascorbic acid dissolved MnO_2 quickly. These rates of dissolution were similar to those observed for catechol and other molecules believed to comprise the core structure of humic substances in natural waters and sediments. The results of our study showed that MnO_2 redox gel probes responded quantitatively to the presence of increasing concentrations of ascorbic acid.

Until recently, direct contact between a reducing enzyme and its solid substrate has almost always been believed to be required for direct enzymatic bacterial reduction (Ehrlich 1990), in which case the redox gel probe technique is unlikely to take this potential activity into account. The internal pore structure of agarose has been reported to range in diameter from 0.1 to 1.0 μm, with even lower porosity developing at the agarose surface (Bassi et al. 1987), and thus likely to exclude most bacterial cells. Laboratory experiments revealed no penetration of the gel probe agar matrix after incubation for 1 month with *Pseudomonas aeruginosa* in nutrient broth at room temperature (data not shown). Thus, redox gel probes principally assess the influence of diffusable by-products of microbial metabolism on particle dissolution. Using a similar approach, Schweisfurth (1968) screened isolates of soil bacteria for Mn (IV) reduction potential by looking for cleared zones around colonies growing on an agar medium containing glucose and solid MnO_2. In this case, the reduction and dissolution of MnO_2 was apparently due to the release of reducing compounds by the microbial colonies or the generation of acid from the metabolism of glucose in the medium. Recently, the dissolution of poorly crystalline Fe(III) oxides immobilized in microporous alginate beads by bacterially reduced electron-shuttling compounds has been shown by Nevin and Lovley (2000), and the possible importance of such indirect dissolution mechanisms in situ is beginning to be considered. The simultaneous incubation of MnO_2-coated rods or slides (Aller and Rude 1988) and gel probes in sediments might allow a distinction between the importance of direct bacterial activity and other environmental factors. Experiments carried out in the present study comparing MnO_2 in gel probes to MnO_2 attached to the surface of plastic rods showed no apparent differences in the depths to which solid MnO_2 remained stable in the wetland sediment (data not shown).

In conclusion, redox gel probes can provide relatively rapid qualitative information about the cycling of redox-sensitive elements within wetland sediments. The gels are made of inexpensive materials and can be deployed rapidly and extensively in the field. These qualities make them ideal for use in mapping biogeochemical activity along transects or in grids within a wetland system. Many useful applications of the technique can be envisioned, such as the evaluation of regions of stability for specific metal sulfides in the tailings ponds of mining operations or in soils or sediments undergoing bioremediation. An approach to evaluate environmental toxicity or biogeochemical activity in sediments based on the immobilization of viable microorganisms in gel probes has recently been proposed (Edenborn and Brickett 2001).

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